Irrdb breeding group workshop

Bahia, Brazil / PMB, 4-7 April 2011

Workshop welcomed by Michelin company at « Plantation Michelin Bahia » (PMB)

Rubber genetics and breeding at Cirad-France Country report of activities from 2007 to 2011 (AGAP research unit)

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Contents

Abstract

In Cirad and the research unit « AGAP », the two groups EGV (Marc Seguin et al.) and BURST (Pascal Montoro et al.) work on rubber genetics and molecular physiology respectively. Whereas BURST group is related with the IRRDB biotechnology group, EGV is more closely associated with the IRRDB breeding group. The main rubber research activities of EGV group are the development of molecular genetic markers for application to rubber breeding including clonal identification, genetic diversity analysis, and QTL-mapping. QTL-mapping was notably applied to the analysis of genetic determinism of SALB resistance sources in Wickham x Amazonian families (CMB = Cirad-Michelin-Brazil project), and extended to a Wickham x Wickham family for the study of growth, latex production and molar mass distribution of rubber chains in native rubber. In the CMB project, SSH banks were created for the generation of ESTs and candidate gene identification, from which a new series of EST-SSR markers were produced. In Brazil, the CMB project also develops a conventional programme of creation and selection of clones, either productive and tolerant to SALB, or adapted to supoptimal areas. Hevea x Corynespora interactions are studied with view to develop resistance breeding. In partnership with IFC (French Rubber Institute), a collaborative network of Large Scale Clonal Trials (LSCT) was developed for characterizing rubber clones, including the IRCA clones issued from a CNRA-Cirad cooperation in Côte d'Ivoire, thus allowing the up-dating of clonal recommendations in Africa.

Introduction

This report is presented as an up-dating of the « Cirad rubber breeding country report » presented at the Irrdb Breeding and Physiology Workshop in Bali (11-12 June 2007).

Both the IRRDB breeding group and biotechnology group deal with rubber genetics. Similarly, at Cirad, there are two groups dealing with rubber genetics and development of improved plant material. The two groups belong to the same « joint research unit », with its former name « DAP », which was enlarged and renamed « AGAP » (Amélioration Génétique et Adaptation des Plantes Méditerranéennes et Tropicales / Genetic improvement and adaptation of Mediterranean and Tropical Plants). This research unit is based on a common biotechnology platform, and it also includes researchers from other French institutions such as Inra (National institute for agronomic research) and SupAgro (National high school of agronomy in Montpellier). This multi-institutional type of « joint research units » (« Umr » = Unités mixtes de recherche) is being developed in France since ten years.

The BURST (Cellular & Molecular Biology of Stress Responses in Tropical Woody Species) group directed by Pascal Montoro (with Julie Leclercq and Olivier Monteuuis), works on in vitro multiplication of rubber (including clonal rootstock development in cooperation with IRRI), genetic transformation, transcriptomics, gene identification. Functional analysis of genes involved in the ethylene and jasmonate biosynthesis and signalling, and the ROS- (Reactive oxygen species) scavenging systems is currently carried out. For instance, plants of two transgenic lines overexpressing the CuZnSOD (superoxide dismutase) were shown to have a better growth and a higher tolerance to abiotic stresses. Besides, transcription factors of the AP2/ERF superfamily were recently identified from a transcript sequence database using the new sequencing technology GS-FLX. The allelic variation of these target genes will be a source of polymorphism of sequence useful for genetic analysis. (see Irrdb biotechnology group, annual report 2009).

The EGV (Evaluation, Management, and Exploitation of Genetic Resources) group directed by Marc Seguin (with Dominique Garcia, Vincent Le Guen, Valérie Pujade-Renaud and André Clément-Demange) is oriented towards rubber breeding, with particular emphasis on molecular breeding based on the generation and use of varied types of molecular genetic markers. This part of activites is presented hereafter.

Actually the differentiation between both types of activities is often very thin, especially if we consider the evolution of tools and techniques. For instance, PCR is used for quantitative analysis of genic expression (qPCR), and for the use of SSR markers as well (microsatellites). And the development of EST-SSRs also gets the breeders in connection with transcriptomics. Now the fast evolution of sequencing techniques also becomes a common ground for (molecular) breeders and physiologists, with the development of new markers (SNP), of genotyping through sequencing techniques, and of the identification and analysis of genes.

Concerning rubber phenotyping at field level, cooperation between physiologists and breeders is still a challenge. Physiologists apply rather sophisticated measurements to small numbers of plants and very few genotypes. This is necessary for analysing such complex traits as rubber response to water stress or to chilling injury. By contrast, breeders need « high-throughput » phenotyping techniques that can be applied to large samples representative of genetic populations. For this cooperation between breeders and physiologists, we have a partnership with Umr « Piaf » (Clermont-Ferrand) which is specialized in tree ecophysiology (water stress and vulnerability to embolism, assessment of chilling injury, sucrose transporters from apoplam to latex cells) and with Umr « Eco& Sols » (Montpellier and Thailand).

The fast evolution of new techniques brings new promises to rubber breeders but also questions. Maybe more than in other plants, rubber breeding is submitted to limits in funding, and these limits vary a lot among the existing breeding teams. Which new techniques can be developed in rubber for use by all the teams with reasonable means, and which other techniques will be more limited in their development due to their cost ?

As for a tree crop, rubber is submitted to the long duration of the breeding process. Field experiments often are a bottleneck to the production of results. Starting from germplasm collection and assessment and going to the development of new performant varieties, rubber breeding may appear as a huge undertaking. Its specificity is clonal selection which allows efficient harness of genetic variability (excepted for rootstocks which still stay almost unselected). There is a request for simplification, and notably for shortening the global duration of the three-step selection pattern (nursery, small scale, and large scale assessments). Actually genetic progress (per time unit and cost unit) is a trade-off between time, funding and accuracy. For providing a simplified view, a rubber breeder does two things : a) choosing parents for generating full-sib families by hand pollination, and b) selecting individual genotypes among and within the families. If we focus on elementary operations, a new rubber variety (budded clone) is the result of the choice of two parents, the creation of one full-sib family, and the identification of one individual genotype. How to improve efficiency in each separate task ? And how to cooperate between teams from different countries without generating too many constraints ? We hope that our activities will contribute to provide breeders with new tools adapted to their constraints and able to allow for the production of new commercial rubber clones adapted to future stakes.

1. Genetic diversity in *Hevea brasiliensis* species

As added to the many contributions of Cirad to field assessment and genetic diversity analysis of *Hevea brasiliensis* species, Le Guen et al. (2009) presented a new assessment of the genetic structure of Amazonian populations of *Hevea brasiliensis* issued from the IRRDB'81 collection. Taking into account the knowledge from a reference genetic map of rubber, 18 SSR markers were chosen so that they were distributed among the 18 chromosomes of the *Hevea* genome (one marker per chromosome). This study, together with Chevallier (1988), Besse

et al. (1994), Seguin et al. (2003), Lekawipat et al. (2004) contributes to the following synthesis.

Due to allogamy, *Hevea brasiliensis* accessions are highly heterozygotic. A huge difference in latex production was found between the domesticated Wickham population and the IRRDB'81 germplasm (in a range from 1 to 10). Field scoring of architectural observations (abundance of branching) indicated a clear differentiation between on the one hand accessions from Acre and Rondonia (poor branching and tall trees adapted to fast access to light in the Amazonian forest), and on the other hand accessions from Mato Grosso which looked more similar to Wickham clones (abundant branching).

One first diversity study of rubber germplasm, based on morphological measurements (Lesprit 2004), showed some differentiation of Acre origin, and a predominance of genetic variability within rather than among the collection sites.

Differentiation of Mato Grosso origin was observed in diversity studies based on isozymes. District RO/PB from Rondonia origin was clustered with Mato Grosso, and a distinction was made between on the one hand West-Acre (AC/T and AC/F), and on the other hand East-Acre and Rondonia. Moreover results from isozyme studies showed that MDF origin was clustered with East-Acre+Rondonia, Schultes-Calima origin was clustered with West-Acre, and Schultes-Palmira was closer to Mato Grosso. As those Schultes-Palmira accessions had a higher production than other wild accessions, it was assumed that they are Wickham x Amazonian hybrids. RFLP studies confirmed those results and district MT/VB was clustered with Rondonia.

The study of rubber cytoplasmic genome revealed only 2 genetic patterns in the chloroplastic genome as opposed to 126 patterns in the mitochondrial genome. The less frequent chloroplastic pattern was specific to East-Acre and partially to district RO/C.

SSR study of rubber germplasm genetic diversity showed a distinction between Wickham, Acre, Rondonia and Mato Grosso origins (Lekawipat et al. 2003). It was continued with the use of 18 SSR markers distributed over the 18 chromosomes of Hevea haploid genome (Le Guen et al. 2009). Diffentiation among populations was estimated as moderate. The main effect of the large geographical distance between origins on differentiation was shown (isolation by distance). A 1720 km distance exists between extreme origins (AC/T in the West, and MT/IT in the East of the Amazonian area). This study confirmed a first level of clustering between on the one hand Mato Grosso and on the other hand Acre+Rondonia+MT/VB+MDF, and a second level of clustering between Acre+MDF and Rondonia. District RO/PB was shown to include a substantial admixture from Mato Grosso origin. The distinction between West-Acre and East-Acre was not really confirmed in this study. A set of hybrid Wickham x Amazonian clones appeared as neighbouring Mato Grosso.

As a conclusion, some rather large diversity would exist within each district of collection, thus complying with the allogamous characteristic of Hevea. Differentiation between sub-populations of Hevea Amazonian germplasm was

found moderate, indicating few limitations to gene flux along the recent evolution apart from genetic distance. The relationship observed between differentiation and the distinct hydrographic networks in the Amazonian area would explain the observed similarity between Mato Grosso and the Wickham population which is located downward of rivers flowing from Mato Grosso.

2. Breeding for tolerance to SALB

Whereas all the productive Asian clones issued from the domesticated Wickham population are highly susceptible to SALB, there are some wild Amazonian genotypes with good level of resistance to SALB but with very low yields. Many attempts were carried out for transfering resistance genes into high-yielding genotypes through back-crossing, but when the new clones were developed, it appeared that resistance was by-passed sooner or later, thus indicating non-sustainable resistance systems which were assumed to be monolocus.

The Cirad-Michelin-Brazil project (CMB) was developed for analysing genetic resistance to SALB in rubber, creating and selecting rubber clones with sustainable resistance to most isolates of Microcyclus and economically acceptable level of latex production. This project, also including cooperations with Brazilian universities, Universidade Estadual de Santa Cruz (UESC, Bahia), and Universidade de Campinas (Unicamp, Sao Paulo), is made of three components :

- Analysis of Hevea x Microcyclus interactions, biology and epidemiology of the fungus (not presented here).

- Analysis of the genetic determinism of different sources of rubber resistance to SALB. This part includes the two complementary approaches of QTL mapping and of identification of candidate genes differentially expressed during the infection by the disease

- Development of plant material through hand pollination and selection.

2.1. QTL-mapping analysis of rubber tolerance to SALB

2.1.1. Genetic analysis of the F1 family PB260 x RO38

Breeding for tolerance to SALB was the research to which the first QTL-mapping approach in rubber was developed by Cirad (Lespinasse et al. 2000a, 2000b) with RFLP markers. It was then continued with SSR and AFLP. It was based on the family PB260 x RO38 for analysing resistance to South American Leaf Blight (SALB) caused by the fungus *Microcyclus ulei*. Phenotyping consisted in the observation of symptoms after artificial inoculation under controlled conditions. On the RO38 parental map, the main QTL, located on the linkage group g13, was detected for five different strains of *Microcyclus ulei*. For all the detected QTLs, the favourable allele (providing resistance) was inherited from F4542, the *H. benthamiana* parent of RO38, and none was inherited from the Wickham *H. brasiliensis* parent of RO38. On the PB260 parental map, only one minor QTL was

identified on the linkage group g15, with the moderately pathogenic strain G70), and it explained only 9% of the variance of the trait. On the consensus map, the QTL analysis with the five strains allowed for the identification of six QTLs on the linkage groups g6, g10, g12, g13, g15, and g16. QTLs were detected on g10, g12, g13, and g15 in the same position as that observed on the RO38 map (Lespinasse et al. 2000b).

Le Guen et al. (2003) extended this phenotyping to field conditions in French Guiana. The major QTL located on linkage group g13 was detected again on the RO38 map, and it was responsible for 36 to 89% of the phenotypic variance of the studied resistance/susceptibility trait. Other minor QTLs were also detected (four in the RO38 map on the groups g2, g8, g13, and g14, and one in the PB260 map on the group g9). By continuing the study of other isolates on the same cross, Le Guen et al. (2007) found that only one QTL contributed to the partial resistance against a highly pathogenic isolate, and no QTL was detected for resistance against the most pathogenic isolate. As an unexpected result, a single isolate could thus completely bypass this polygenic resistance. Enlargement of this research to « association genetics » through the study of « linkage desequilibrium » was carried out on some portions of chromosomes in Amazonian natural populations (Le Guen 2008).

A preliminary study of the family PB260 x FX2784 showed the existence of a major resistance gene on linkage group g2, different from those found in RO38.

2.1.2. Genetic analysis of the F1 family PB260 x MDF180

MDF180, although with a rather low yield, appeared to exhibit a partial but durable resistance to SALB for more than 30 years and on a large planted area. The segregating family PB260 x MDF180 was used for analyzing the genetic determinism of MDF180 resistance. Results were presented recently by Le Guen et al. (2011). A genetic map of this family was built, based on SSR and AFLP markers. Phenotyping was carried out in both controlled conditions with three different Microcyclus isolates (2 from Bahia, and 1 from French Guiana), and under natural infection in a field trial in French Guiana. As no QTL was found in PB260, further QTL detection was therefore carried out only on the MDF180 genetic map. A total of 6 QTLs were identified, two of them having a major effect. One QTL located on linkage group g15 appeared as probably resulting from a major gene for qualitative resistance (*M15md*), conferring a complete resistance to natural infection in the Guiana field trial as well as to controlled inoculation by the Guiana isolate (qualitative resistance locus). By contrast, *M15md* had only a minor effect with the two isolates from Bahia. Another QTL detected on g13 had a high effect after controlled inoculation by the two isolates from Bahia. This QTL on g13, not efficient on Guianese isolates, would be different from the major QTL of resistance found in RO38. Four minor QTLs were also detected. Two of them were detected in the Guiana field trial, whereas the two others were detected in controlled conditions for one of the two isolates from Bahia. As a result, these QTLs show specific effects depending on the isolates from two different areas

Research of new genetic factors of resistance to SALB is currently continued on the family PB235 (susceptible) x FDR5597 (resistant). Such an exploration of the

diversity of resistance genetic factors can help in developing the pyramiding of complementary resistance genes and selection of rubber clones with general tolerance to most of *Microcyclus* isolates. For each of the three studied resistant parents (RO38, MDF180, and FDR5597), it was found in each case that resistance was mainly depending on one or two major genes or QTLs, and not on a number of different QTLs with cumulated small effects.

2.2. Detection of candidate genes by SSH approach

Whereas the genomic markers used in the QTL approach were used based only on linkage disequilibrium, and had no functional link with resistance genes, it was also endeavoured to identify the genes differentially expressed during infection and the markers included in the sequences of these genes (EST-SSR, SNPs). Therefore transcriptomics analysis was applied to the Hevea/Microcyclus interaction (Cirad Montpellier, and UESC Ilheus Bahia).

By use of Suppressive Substractive Hybridization, SSH banks were built for two couples of susceptible vs resistant varieties (PB260 x RO38 at Cirad, and PB314 x MDF180 at UESC), and the expression patterns were analysed in the course of the infection process after controlled inoculation of leaves by the disease, either by macro-array or quantitative PCR (q-RT-PCR).

This SSH approach led to the cloning of 6858 unigenes associated with SALB infection, the characterization of 489 candidate genes selected for their differential expression along the infection process, and the identification of 125 new EST-SSR molecular genetic markers which contributed to a higher density of the genetic maps in the QTL-mapping approach.

2.3. Identification of 13 productive and SALB-tolerant CMS clones

Today a set of 13 CMS clones (Cirad-Michelin Selection) seem to offer a good level in both resistance and production, and they are currently disseminated for evaluation in Large Scale Clonal Trials for assessment in multilocal conditions. These clones are : CD1174, CDC56, CDC312, FDR4575, FDR5240, FDR5283, FDR5597, FDR5665, FDR5788, FDR5802, MDX607, MDX624, PMB1. Three of these clones, CDC312, FDR5788, and PMB1 are currently recommended and developed in Brazil.

2.4. Hand pollination and development of clones

From 1993 to 2009, hand pollination was carried out each year in Plantation Edouard Michelin (PEM) in Mato Grosso (Brazil) for the production of new families and the selection of new clones of two types :

- productive and SALB-tolerant clones
- clones adapted to sub-optimal areas.

Depending on the parents, different types of F1 families were created: a) productive x productive and productive x weak resistance, among which new clones were selected for adaptation to suboptimal areas, b) productive x sustainable resistance among which new clones were selected for development in SALB-affected areas, and c) crosses with the pyramiding of two sources of sustainable resistance for selection of new parents.

A classical three-step selection pattern (with Seedlings Evaluation Trials, Small Scale Clonal Trials, and Large Scale Clonal Trials) was used for selection of CMB clones among and within the families. Depending on the objective, selection was carried out in Bahia, under SALB pressure, or in the suboptimal ecological conditions of the South of Mato Grosso State of Brazil.

3. Growth and latex production in the Wickham population

Cooperation with Rrit-Doa in Thailand allowed us to develop genetic mapping based on non-expressed genomic SSR markers (as opposed to genic EST-SSR markers inserted in the sequences of expressed genes) and QTL-mapping on the experimental F1 family RRIM600 x PB217 from 2002 to 2009 (Genmap project). This research was applied to growth, latex production, and the distribution of molar mass which is related with the quality of native rubber (publications in preparation). Among 48 QTLs detected, most of them with small effects (explaining from 5 to 15 % of phenotypic variance), 2 important QTLs with large effects were mapped, one for growth in girth of the trunk during favorable growing periods (in rainy seasons), and the other for latex production.

A similar approach is currently being developed by CBMEG (University of Campinas in Brazil) on the family PR255 x PB217, in cooperation with Cirad and Michelin company.

Results from the Genmap project suggest different ideas :

- The stability of the two QTLs should be validated by assessment on another set of genotypes issued from the same family and phenotyped in the same ecological site
- QTL detection in this same family should be carried out again in another ecological site for testing stability among the variations of the environment
- MAS based on the two important QTLs should be tested in other F1 families
- Although SSR genotyping is still rather expensive, a plan for marker-assisted selection (MAS) might be developed on some large-sized families mainly based on these two QTLs
- The improved accuracy which is hoped from MAS might be combined with a new approach of early selection in rubber, by merging the two steps of selection in nursery and in small scale clonal trial.

Experimental populations used for QTL detection in CP (« cross-pollinated ») species like rubber are F1 progenies issued from two heterozygous parents, and therefore the same full-sib families which are usually created in rubber for clonal

selection of individual genotypes. Frequently breeders generate many small-sized families and develop some empirical family x individual selection on seedlings at nursery stage. Simmonds (1996) insisted on the importance of « family selection in plant breeding », but actually, literature is very poor about the comparison of families in rubber. In fact, such family comparison may be difficult and inaccurate due to the frequent small size of the families and probably also to the fact that within-family variation seems higher than between-family variation. Those elements suggest that it might be more profitable to explore large-sized F1 progenies for a limited number of families with specific interest, which would justify some added investment in the use of genetic markers for QTL detection and MAS. A combined use of genetic information and phenotypic measurements might provide a better accuracy for estimating genetic values, and as a result, a better selection efficiency.

4. Clonal identification by SSR markers

Genomic SSR markers, monolocus and codominant, are characterized by their very high polymorphism based on the large variation of the number of di-, tri-, or tetra-nucleotide repeats among alleles (generally more than 10 alleles per locus). As a result, the combination of a small number of SSR markers is enough for determining unique patterns of a large number of clones.

Based on a set of 8 SSR markers, Cirad has built a reference file of distinct patterns for around 600 different rubber clones from varied origins (Wickham, Amazonian, W x Am, non-brasiliensis species). This tool is routinely used for checking the identity of plant material in research as well as in development, notably for checking clonal conformity in budwood gardens. As far as the vegetative multiplication of rubber varieties is prone to many mistakes concerning the identity of the propagated varieties, this method is a very valuable tool for increasing quality control in rubber multiplication, and a real application of biotechnological research to rubber cropping development.

Table : Name, access number, number of alleles and PIC (Polymorphism Information Content) of the 8 SSR markers used by Cirad for clonal identification. Nb of alleles and PIC were calculated from a set of 500 genotypes including Wickham and Amazonian clones.

Marker	SSR name	Genbank access n°	Nb alleles	PIC
HB01	M421	AF383935	17	0.802
HB02	A31	AF383940	25	0.882
HB03	M124	AF221697	21	0.849
HB04	MnSod	G73377	25	0.888
HB05	T65	AF383942	17	0.839
HB06	M574	AF221706	27	0.921
HB07	M127	AF221698	16	0.829
HB08	A66	AF383941	20	0.897

The international system for certification of plant varieties is UPOV (Union pour la Protection des Obtentions végétales - International Union for the Protection of New

Varieties of Plants). This system admits only morphological traits for variety recognition, and isozymes are accepted only as complementary traits.

For a tree like rubber, observing morphological traits on living plants is long, difficult, and prone to many mistakes and uncertainties. By contrast, analysing SSR markers from leaf samples taken from budwood gardens is very powerful and accurate. Even if such SSR analysis implies the use of a specialized laboratory, developing this method might be a fast and simple way for setting a IRRDB service of certification of rubber clones, based on the expertise of two or more independent laboratories, and on the use of publicly available genomic resources.

5. Study of Hevea x Corynespora interaction

Since its first identification in rubber in 1958, the Corynespora Leaf Fall Disease (CLFD) due to the necrotrophic fungus *Corynespora cassiicola* fastly spread to many rubber cropping areas in Asia and Africa. It is considered as an important threat to rubber since the outbreak in Sri Lanka in 1985, which made necessary the felling of 4500 ha of the clone RRIC103 in this country. By contrast with Colletrotrichum or Microcyclus, which develop on leaves only until stage B, Corynespora can develop on mature leaves at stage C. Rubber breeding for tolerance to this disease is considered.

Corynespora seems to develop more in favorable areas of rubber cropping with a high moisture and short annual dry periods, close to Equator line. From our experience, Corynespora outbreaks seem to develop on young plantations at the time when canopy is closing, just before the initiation of tapping (from 4 to 8 years of age), notably when a susceptible clone has been planted on large areas and approaches this age. Outbreaks seem to last only a few years, just as if the trees were becoming more resistant with age. This seemed to be the case in South Cameroon from 1989 to 1993, and in North Sumatra from 1998 to 2002. By contrast, Corynespora outbreaks on the clone IRCA18 in Nigeria were first observed in 2000, and currently still persist.

RRIM600 was very susceptible in the South of Malaysia and North-Sumatra. IAN873 suffered from hard Corynespora attacks in North Sumatra (Eschbach 1992). PB260 was very susceptible in South-Cameroon (from 1989 to 1993). PB217 was found susceptible in South-Cameroon, Nigeria, and North-Sumatra. RRIC110 was found susceptible in Côte d'Ivoire in 1990. RRIC121 was found very susceptible in Nigeria since the first observations in 1994. RRIM712 was found very susceptible in North-Sumatra. RRII105 was found susceptible in Sri Lanka and India. IRCA18 was found very susceptible in Nigeria since 2000 and in Ghana since 2005.

GT1 and AVROS2037 are considered rather tolerant to Corynespora. From our observations, RRIC100 was found as the clone most tolerant to Corynespora in plantations, although its susceptibility to CCP isolate (from Philippines origin) was shown by controlled infection in laboratory. This clone seems one of the best solutions in Corynespora-affected areas.

The production of the toxin cassiicolin by Corynespora fungus was first evidenced by Onesirosan (1975), and Sarma and Nayudu (1975). Research about the action of this toxin was developed by Breton et al. (2000). Cassiicolin would be a host-selective toxin. The range of hosts appeared similar for the toxin and for the fungus (Breton 1997 ; Barthe et al. 2007). Tests of phytotoxicity of CCP isolate on susceptible vs resistant clones showed a high susceptibility of the susceptible clone to Corynespora culture filtrate (toxin with no fungus), and a correlation was found between clonal susceptibility to the fungus and susceptibility to culture filtrate. Other experiments showed the major role of cassiicolin in Corynespora pathogenicity (Breton et al. 2000). Cassiicolin was purified (Lamotte et al. 2007), which allowed for the protein sequencing and the characterization of its 3D structure by Nuclear Magnetic Resonance. Cassiicolin is a small glycoprotein of 2.8 LDa, with 27 aminoacids and 3 disulfur links.

A large morphological and physiological diversity of Corynespora isolates was shown, but no correlation was evidenced between morphological diversity and pathogenicity. A genetic variability of rubber clones was shown for resistance to one same isolate, and conversely, a variation of intensity of the disease was shown among isolates for one same clone (Breton et al. 2000). The genetic diversity of the fungus was studied by use of different types of molecular genetic markers (RAPD, ISSR, rDNA-ITS).

Moreover, variations in the level of resistance/susceptibility of some clones with planting sites were reported, suggesting the possible existence of Corynespora races, each having a specific range of hosts. The existence of two races were shown by Nghia et al. (2008). Research at Cirad is now focussed on the exploration of a large diversity of isolates for the identification of distinct Corynespora races and the analysis of interactions between these races and rubber clones.

6. Development of IRCA clones

IRCA clones were created and selected in Côte d'Ivoire, from 1972 to 2001, through a cooperative programme of Cirad (France) and CNRA (Côte d'Ivoire).

Assessment of rubber clones in large scale clonal trials (LSCT) is carried out in cooperation with the IFC, an association of European companies involved in tropical agro-industry (Institut Français du Caoutchouc / Rubber French Institute), including Michelin, Socfinco, and Siph, on rubber estates located in Africa and Brazil.

The main clones studied in Africa are :

- PB217 and RRIC100 (class 1)

- a set of the best IRCA clones : IRCA41, IRCA101, IRCA109, IRCA230, IRCA317, IRCA331, IRCA427, IRCA523, and IRCA840 (class 2)

- a set of clones which are considered as performant but hazardous due to well-identified drawbacks : IRCA18, IRCA130, IRCA733, IRCA804, PB235, PB255, PB260, PB312, PB314, PB330, and RRIM703 (classe 3a)

- a set of medium clones for diversification : GT1, RRIM600, PR107, RRIM712, PB254, PB280, PB324, RRIC102, RRIC121, and IRCA clones : n° 15, 19, 22, 27, 120, 122, 144, 145, 209, 229, 321, 323, 428, 430, 440 (class 3b).

Clones recommended by Cirad to smallholders in Africa are : RRIC100, PB217, IRCA41, IRCA230, IRCA317, IRCA331.

Table in appendix shows the adjusted mean cumulated dry rubber productions (kg/ha) of clones studied in 44 LSCT of Côte d'Ivoire and Nigeria. Even if this table provides a comparative perception of the performances of many clones in the ecological context of these two countries, it does not give any accurate and realistic view of the advantages and drawbacks of each clone, notably in relation with wind damage, TPD, and Corynespora susceptibility. For instance, even if PR107 appears as low yielding in the short and medium run, it is able to maintain a high density of tapped trees over a very long time and this clone is still appreciated and moderately planted in Africa due to its high production level in the long run

PB217 is promoted by Cirad as a very important slow-starter clone, able to show important response to stimulation after 4 or 5 years of tapping and in the same time to maintain a fast growth during tapping and a good tapped stand along time (low susceptibility to wind damage and TPD). The first positive result for this clone was observed in a trial planted in 1972 (Côte d'Ivoire), and its high potential was regularly confirmed afterwards, in trials as well as in estates. In the older trial with this clone, it achieved a cumulated production of 50 tons per ha at 27 years of age (as compared to 44 tons for PB235, 40 tons for RRIM600, 37 tons for GT1, and 35 tons for AVROS2037). Although suceptible to leaf diseases, it could rank first for cumulated production in South-Cameroon affected by Colletotrichum and Corynespora outbreaks for many years. The potential of this clone would be related with its high level of sucrose content in the latex, as a possible result from a high efficiency in sucrose transport from the apoplasm to latex cells (Dusotoit-Coucaud et al. 2009). However this clone has also drawbacks such as a low budding success rate, a rather slow growth before tapping (equivalent or slower than GT1), susceptibility to Colletrotrichum and Corynespora, poor mechanical stability of the latex (it cannot be used for centrifugation), and low PRI (plasticity reduction index). Apart from that, its native rubber (just issued from trees) has typically a monomodal distribution of molar mass, as opposed to RRIM600 which is typically bimodal (for this concept, see Subramaniam 1976). We promote the use of PB217 as a parent in crosses, but due to its very low female fertility, it is a bad seed-parent and can be used only as a male.

RRIC100 was promoted to class 1 in our classification for Africa, due to its high tolerance to Colletrotrichum and Corynespora in the field. It must be noticed however that it was found susceptible to some Corynespora isolates in the controlled conditions of laboratory studies. This clone, with a medium-high latex

production, is also appreciated for its fast growth before tapping, thus allowing early tapping (9 months earlier than GT1). Budding success rate may be low.

PB260 was formerly appreciated as a quick-starter, but it was taken out of our class 1 and put to class 3a (hazardous clones) due to its high susceptibility to wind damage and TPD.

IRCA18 was taken out of our class 2 and put in class 3a (hazardous clones) due to its high susceptibility to Corynespora (higher than PB260), observed in Nigeria and Ghana.

Many clones were put to class 3a (hazardous clones) due to their susceptibility to wind damage, observed during recent years (IRCA130, IRCA733, IRCA804, PB235, PB260, PB312, PB314, PB330). RRIM703, although a quick starter with high initial yield, was found very susceptible to TPD and Colletrotrichum, and probably prone to wind damage (important benting of the trunks along time).

The most promising IRCA clones (IRCA41, IRCA101, IRCA109, IRCA230, IRCA317, IRCA331, IRCA427, IRCA523, and IRCA840), although developed on estates only on some hundreds or a few thousands hectares so far, still appear very good. Like PB217, IRCA41 seems to maintain a high level of sucrose in its latex and would respond positively to intensification by stimulation (it would be susceptible to Oïdium). IRCA109 showed some signs of susceptibility to wind damage. IRCA317 is a quick-starter. IRCA230 (although susceptible to Corynespora) and IRCA331 would be the most performant clones.

Table : Semi-quantitative scoring of IRCA and some Asian clones, from assessment in Africa. Calculation of a global score (maximum = 100). The seven first clones (from GT1 to RRIM600) are used as a reference for assessing the other clones.

Variable	**G**	**P15**	**P1525**	**RV**	**TPD**	**Col**	**Cor**	**DL**	**Bois**	**Gref**	**Oid**	**Tech**	**Score**
Max score	**5**	**35**	**15**	**10**	**7**	**5**	**5**	**6**	**5**	**1**	**2**	**4**	**100**
GT1	2	15	10	5	4	0	4	4	2	1	1	2	50
PB217	1	18	15	9	7	1	2	6	3	0	1	0	63
PB235	5	35	5	0	0	3	2	0	5	1	0	3	59
PB260	3	32	5	0	0	5	1	0	4	1	1	3	55
PR107	0	1	15	10	7	2	3	4	3	1	1	4	51
RRIC100	4	18	10	5	4	5	5	3	4	0	1	3	62
RRIM600	1	15	10	7	4	3	3	2	0	1	2	3	51
IRCA18	2	30	10	6	5	1	0	1	3	1	1	2	62
IRCA19	4	17	11	3	7	4	2	5	4	1	1	2	61
IRCA41	3	17	14	7	7	1	2	5	3	0	0	2	61
IRCA101	3	25	12	6	3	2	2	3	3	1	1	2	63
IRCA109	3	30	10	4	5	2	2	4	4	1	1	2	68
IRCA145	3	25	8	4	2	2	2	2	2	1	1	2	54
IRCA209	3	25	8	5	3	2	2	1	2	1	1	2	55
IRCA230	4	32	10	5	4	2	1	3	4	1	1	2	69
IRCA317	4	33	10	5	1	2	2	1	4	1	1	2	66
IRCA323	2	19	11	6	2	2	2	5	3	1	1	2	56
IRCA331	2	32	12	6	4	2	2	5	3	1	1	2	72
IRCA427	2	24	12	6	6	2	2	3	2	1	1	2	63
IRCA428	3	25	12	5	6	2	2	4	2	1	1	2	65
IRCA523	4	25	12	5	1	2	2	2	3	1	1	2	60
IRCA631	2	17	10	1	4	2	2	3	4	1	1	2	49
IRCA733	4	28	8	3	1	2	2	3	5	1	1	2	60
IRCA804	3	32	5	2	4	2	2	2	4	1	1	2	60
IRCA840	3	29	10	6	2	2	2	2	3	1	1	2	63
PB330	4	31	7	0	2	1	2	4	5	1	1	2	60
RRIM703	2	31	5	2	1	1	2	0	2	1	1	2	50
RRIM712	2	17	13	8	6	0	0	3	2	1	1	2	55

G	growth before tapping
P15	cumulated latex production at 15 years of age
P1525	cumulated latex production from 15 to 25 years
RV	resistance to wind damage
TPD	Tapping Panel Dryness (dry cut and/or brown bast)
Col	Tolerance to Colletotrichum
Cor	Tolerance to Corynespora
DL	Latex diagnostic – Adaptation to intensification by stimulation
Bois	Rubberwood production
Gref	Budding success
Oid	Tolerance to Oïdium
Tech	Quality adaptation to technological uses

Conclusions

From these researches, it was shown in rubber that the use of molecular genetic markers for QTL-mapping was an efficient tool for analysing genetic determinism of traits of interest, with view to Marker-Assisted Selection.

Considering the contribution of genomics to plant breeding, the main trend is the fast evolution of sequencing techniques with fast-decreasing prices. As a consequence, SNP markers will be detected in illimited numbers and SNP genotyping will really become a high-throughput technique. Sequencing will become a routine tool for genotyping, and also for the quantification of gene expression. SNP markers should be the base of association genetics for MAS, in rubber as for most of plants. This might be true in 5 to 10 years from now. Genomic selection, which involves the use of tens of thousands of SNP markers, is more and more presented as applicable to many species in the near future. Application will mainly be a question of cost. Will it be affordable in routine to most of rubber breeding teams ?

By contrast, we now have many hundreds of publicly available SSR markers, and many laboratories are, or become, able to use them for genotyping. Those markers can be currently used for combining methodological research and selection in the same experiments. Although the cost of their application is still rather important, they can be used for analysing the genetic determinism of varied traits in different ecological sites, thus generating new genotypic criteria for assisting in phenotypic selection. If we assume that each breeding team studies one or two families, gathering of informations may become valuable for the whole rubber breeding community. Thus cooperation out of any coordination constraints can be considered.

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Appendix

Table : Meta-analysis of 44 LSCT in Africa (Côte d'Ivoire, Nigeria). Mean data were adjusted by least square method (Sas-Lsmeans procedure). Ranking by decreasing order of A15 (cumulated dry rubber production per ha at 15 years of age).

Clone	A6	A7	A8	A9	A10	A11	A12	A13	A14	**A15**	A16	A17	A18	A19	A20
IRCA331	0	1277	3240	5462	7576	10245	13275	16998	19984	**23467**	26358	29164			
IRCA230	407	1796	4043	6213	8921	11700	14531	17546	20050	**22477**	25307	28277	30839	33019	35175
IRCA317	275	1706	3928	6528	9336	11833	14709	16772	19216	**21448**	23745	26590			
IRCA804	673	2202	4124	6409	9044	11519	14047	16539	18894	**21181**					
IRCA825	329	1654	4165	6503	8940	11369	13693	15902	18191	**20335**	22433				
RRIM703	0	728	2476	4871	7524	10062	12721	15425	17867	**19908**	21825	24107	26137	28346	29729
IRCA523	443	2121	4488	6735	8572	10914	12838	15288	16998	**19654**	22087				
IRCA733	351	1433	2973	4972	7390	9712	12064	14540	17160	**19580**	21739				
IRCA101	129	1489	3491	5660	8041	10386	12799	15117	17467	**19496**	21843	24806			
IRCA840	191	1810	4147	6323	8669	10900	13178	15119	17252	**19339**	21551	24212			
PB312	129	1857	4348	7098	9342	11663	13787	15530	17263	**18981**	21026	23559			
PB235	766	2188	4156	6337	8537	10335	12111	14239	16260	**18835**	20936	23999	25986	28768	31013
PB260	279	1558	3594	5813	8468	10579	12895	14970	16938	**18702**	20803	23731	25676	28074	29871
PB330	112	1193	2839	4872	7289	9386	11920	14220	16341	**18300**	20706	23269	25479		
IRCA109	227	1315	2964	4979	7168	9088	11468	13882	16300	**18158**	20742	23137	24447	26781	28424
IRCA427	222	1375	2907	4776	6778	9025	11361	13760	16046	**18125**	20721	22887			
IRCA18	226	1508	3497	5498	7475	9550	11582	13834	15973	**18105**	20250	23014	25169	27854	30431
IRCA538	666	2550	4471	6524	8578	10414	12282	14378	16155	**18094**	20113	22274			
VM515	108	1090	3072	5485	7894	10031	12112	14321	16695	**18043**	19915	22198			
IRCA145	223	1289	3003	5108	7318	9491	11718	13878	15865	**17864**	19816	22147			
PB310	186	782	2346	4447	6724	9076	11337	13383	15504	**17643**	19569	20474			
IRCA416	0	1007	3363	6058	8438	10846	12661	14134	15887	**17620**	19417	22055			
PB280	222	1391	3619	5373	7343	9160	11326	13336	15579	**17500**	19476	21783			
IRCA631	103	887	2652	4919	7172	9304	11483	13682	15563	**17476**	19590	21765			
RRIC100	328	1209	2685	4426	6347	8490	10862	13128	15248	**17473**	19305	21933	23985	25834	27945
IRCA41	139	1228	2842	4685	6695	8537	10609	12686	14922	**17403**	19561	21785	23916	26465	29349
IRCA209	270	1795	3878	5807	7834	9707	11856	13709	15692	**17380**	19071	20857			

IRCA111	389	1934	3972	6206	8398	10180	11999	13809	15526	**17347**	19201	21142	21903		
IRCA321	206	1155	2668	4561	6758	8892	10928	12909	14926	**16963**	19064	21334			
IRCA130	330	1776	3554	5535	7504	9282	11158	13232	15121	**16936**	19133	21490	22795		
PB324	0	934	2432	3990	6173	8042	10191	12288	14499	**16826**	19263	21687	23764		
IRCA229	140	1540	3297	5274	7289	8902	11173	13155	15260	**16786**	19426	23230			
IRCA27	266	1241	2876	4765	6496	8385	10428	12504	14589	**16743**	18707	21137	23501	26120	28385
PB255	108	1171	2912	4750	6895	8890	11005	13019	14909	**16697**	18522	20966	22742		
IRCA305	693	1926	3407	5218	7290	9164	11042	12879	14807	**16678**					
RRIM712	0	572	2134	3991	6101	8241	10434	12664	14658	**16559**	18901	21271	23146		
IRCA22	279	1525	3452	5248	7131	8774	10534	12329	14187	**16547**	18315	21226	23323	25905	28200
IRCA842	454	1939	3718	5563	7489	9263	10973	12800	14677	**16254**					
PB217	0	570	1598	3115	5008	7048	9364	11602	14047	**16240**	18843	22094	24807	27572	30542
IRCA122	112	683	2007	3791	5903	7867	10084	12272	14350	**16229**	18218	20666	22629		
RRIC102	394	1213	2376	3759	5521	7378	9434	11661	13616	**15909**	17809	20325	22208	24149	26464
IRCA323	0	875	2265	3796	5652	7439	9463	11186	13392	**15739**	18150	21768			
HARBEL60	102	785	2213	3867	5984	7668	9543	11378	13728	**15646**	17962	20420	22393	24945	
IRCA408	261	1114	2087	3292	5009	6876	9044	11099	13408	**15555**					
IRCA202	0	757	2160	3810	5604	7473	9496	11325	13240	**15196**	16931	18729			
IRCA407	0	963	2383	4168	5847	7600	9317	11181	12963	**15076**					
IRCA19	137	1054	2559	4253	6154	8041	9436	11090	12854	**14823**	16573	18401	20550	22899	25455
IRCA814	519	1571	2753	4133	5757	7376	9103	11007	12868	**14796**					
RRIC103	151	425	1217	2171	3192	5154	7153	9814	12032	**14579**	16573	19243	21527	24080	27283
GT1	0	530	1617	3066	4874	6588	8600	10573	12543	**14516**	16492	18846	20749	22924	25049
IRCA303	0	678	1925	3580	5346	7181	9035	10806	12637	**14261**					
IRCA126	0	586	1907	3713	5594	7466	9153	11155	12634	**14165**	15486	17374	18206		
PB254	0	689	1587	2844	4492	6024	8155	10095	11938	**14007**	16453	19346	21514	23251	26128
BPM24	142	825	2322	4054	5750	7373	9096	10766	12344	**13986**	15897	18028			
RRIM600	0	500	1606	2962	4819	6559	8543	10179	11964	**13480**	15555	18112	19927	22412	24440
HARBEL10	0	0	722	2150	3917	6004	7836	9898	11564	**12936**	14071	15468	16675	18003	
AVROS2037	305	1118	2182	3479	5194	6947	8557	10031	11417	**12642**	13987	16419	18112	20201	22263
IRCA144	0	947	2266	3720	5087	6431	7852	9355	10827	**12392**	13093	14615	15498		
Nab17	0	297	1055	2405	4201	6170	8480	10664	11221	**12276**	13544	15115	16823	19043	20796
IRCA307	233	1152	2208	3649	5022	6465	8025	9432	10973	**12253**					

RRIM527	0	0	798	2482	4063	5887	7707	9244	10437	**12243**	12925	14287	15885	17563	18960
PB28/59	0	0	913	2372	4043	5806	7639	9409	10550	**11662**	13054	14930	16330	17874	19471
RRIC101	212	738	1887	3124	4289	5484	6628	8119	9918	**11642**	13449	15764	16990	18921	20475
AF261	0	434	1074	1740	3233	4418	6497	7897	10113	**11468**	14097	16225	18309	20678	22425
RRIM707	0	662	1531	2672	4141	5693	7357	8754	10223	**11261**					
RRIC110	354	1588	3189	5222	7289	8210	8476	9366	10332	**11242**	12396	13636	14171	14931	15967
IRCA723	0	648	1879	3319	4871	6347	7862	8953	10200	**11180**	12090				
IRCA515	0	588	1856	3426	4174	5562	6670	8409	9453	**11116**	11459				
RRIM701	0	695	1724	2782	4214	5669	7395	8870	10093	**10961**					
IRCA117	308	942	1896	2983	4184	5383	6786	8091	9462	**10692**	11684	12693			
IRCA37	0	839	1954	3116	4219	5463	6487	8117	9418	**10689**	11797	13032	14404	16078	17953
IRCA617	0	266	1022	2237	3193	4500	5716	7216	8584	**10135**	11791				
IRCA707	232	1029	2158	3162	4376	5425	6166	7352	8550	**9720**	10643	11735			
PB5/51	112	642	1481	2500	3692	5021	6664	7772	8817	**9641**					
TR1549	0	400	1224	2140	3422	4975	6706	7824	8564	**9095**					
PB86	0	0	306	1016	2266	3506	5084	6030	6790	**8016**					

Table : Meta-analysis of 44 LSCT in Africa (Côte d'Ivoire, Nigeria). Mean data were adjusted by least square method (Sas-Lsmeans procedure). Ranking by decreasing order of A10 (cumulated dry rubber production per ha at 10 years of age).

Clone	A6	A7	A8	A9	**A10**	A11	A12	A13	A14
PB314	154	2089	4850	7488	**9612**	11989	14462	17065	19177
IRCA987	119	1464	3471	5860	**8542**	11166	13195	15506	
IRCA933	119	1460	3493	5826	**8333**	10692	13225	15408	
IRCA909	119	1172	3136	5447	**8012**	10301	13133	15359	
IRCA986	119	1158	3117	5192	**7955**	10739	13879	16472	
IRCA945	119	1042	2813	4878	**7706**	10168	12290	13980	
IRCA15	154	1419	3478	5178	**7660**	9712	12415	14638	17118
IRCA966	119	1053	2862	4779	**7510**	9751	12362	14740	
IRCA959	119	1225	3109	4785	**7454**	9778	12206	14506	
IRCA911	119	1013	2775	4746	**7397**	9534	12031	14534	
IRCA916	119	948	2743	4672	**7120**	9303	11574	13553	
IRCA430	149	1307	3044	4884	**7019**	8693	10717	12240	13807
RRIC121	350	1524	3082	4750	**7013**	8661	10845	12978	15327
IRCA982	119	1268	2868	4707	**6842**	8675	10453	12190	
IRCA428	149	1241	2836	4641	**6731**	8743	11105	13127	15710
IRCA440	149	1286	2862	4561	**6547**	8339	10353	12029	13774
IRCA120	285	1069	2186	4104	**6228**	8432	10641	13131	15270
IRCA919	119	778	2352	4076	**6085**	7673	9168	10435	
IRCA984	119	778	2336	3912	**5853**	7262	8741	9890	
IRCA411	131	737	1695	3033	**4899**	6381	7905	9603	11034
PR228	0	659	1455	2828	**4068**	5214	6618	7728	8880
PB252	0	611	1462	2306	**4022**	5606	7595	9162	
Y427/3	0	407	1142	2332	**3574**	4944	6324	6806	7518
Y3/46	0	483	1103	1952	**3358**	4581	5996	6610	7585
IAN717	0	477	1106	2189	**3195**	4341	5827	6626	7068
PR107	0	274	651	1669	**2940**	4253	5828	6596	7488

End.